Crystal Structure of Agaricus bisporus Mushroom Tyrosinase

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Supplementary Information belonging to

Wangsa T. Ismaya, Henriëtte J. Rozeboom, Amrah Weijn, Jurriaan J. Mes, Fabrizia Fusetti, Harry J. Wichers and Bauke W. Dijkstra. Crystal structure of *Agaricus bisporus* mushroom tyrosinase - Identity of the tetramer subunits and interaction with tropolone.

Fig. S1. *A. bisporus* tyrosinase tetramer in the asymmetric unit of a) space group $P2_12_12$ (dimensions ~139 x 97 x 59 Å), and b) space group $P2_1$ (dimensions ~107 x 87 x 84 Å). The H subunits are coloured red and green, and the L subunits are in light blue and purple. The black balls represent the positions of the bound holmium ions (see main text for explanation).

a) b)

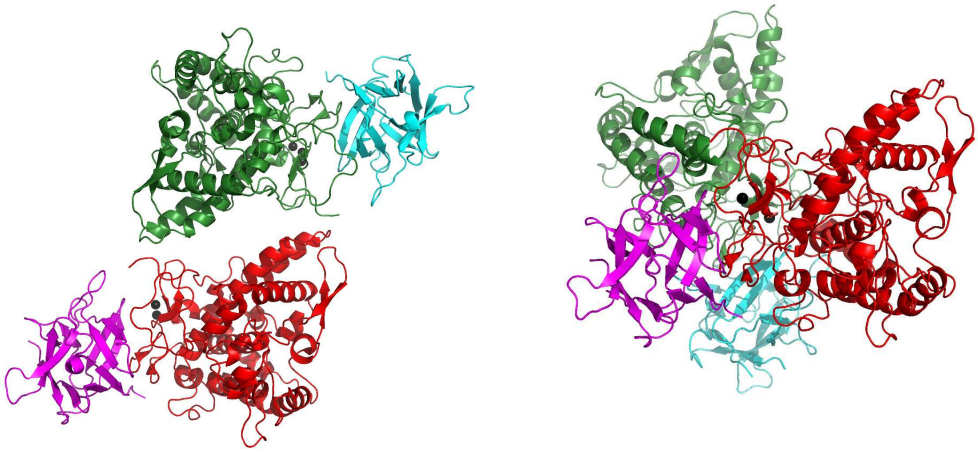


Fig. S2. Structure-based sequence alignment of tyrosinase-related type 3 copper proteins. The aligned structures and PDB database accession numbers are *Ab*Tyr, *A. bisporus* tyrosinase (PPO3, this article; PDB 2y9w); *Bm*Tyr, *B. megaterium* tyrosinase (PDB 3nm8 (*1*)); *Sc*Tyr, *S. castaneoglobisporus* tyrosinase (PDB 1wx2 (*2*)); *Ib*Cox, *I. batata* catechol oxidase (PDB 1bt1 (*3*)) and *Od*hcy, *Octopus dofleini* hemocyanin (PDB 1js8 (*4*)). Structural alignment was performed using the DALI server. The secondary structure elements are shown as squiggles (α-helix and 3_{10}-helix), black arrows (β-strands) and strict β-turns as **TT** letters at the top of

the alignment. Residues involved in copper binding are shown in cyan. Cysteines involved in the thioether bond are shown in purple. The fully conserved residues are shown in white on red background. Numbers indicate the residue numbers of *Ab*Tyr. The figure was prepared with ESPript (*5*).

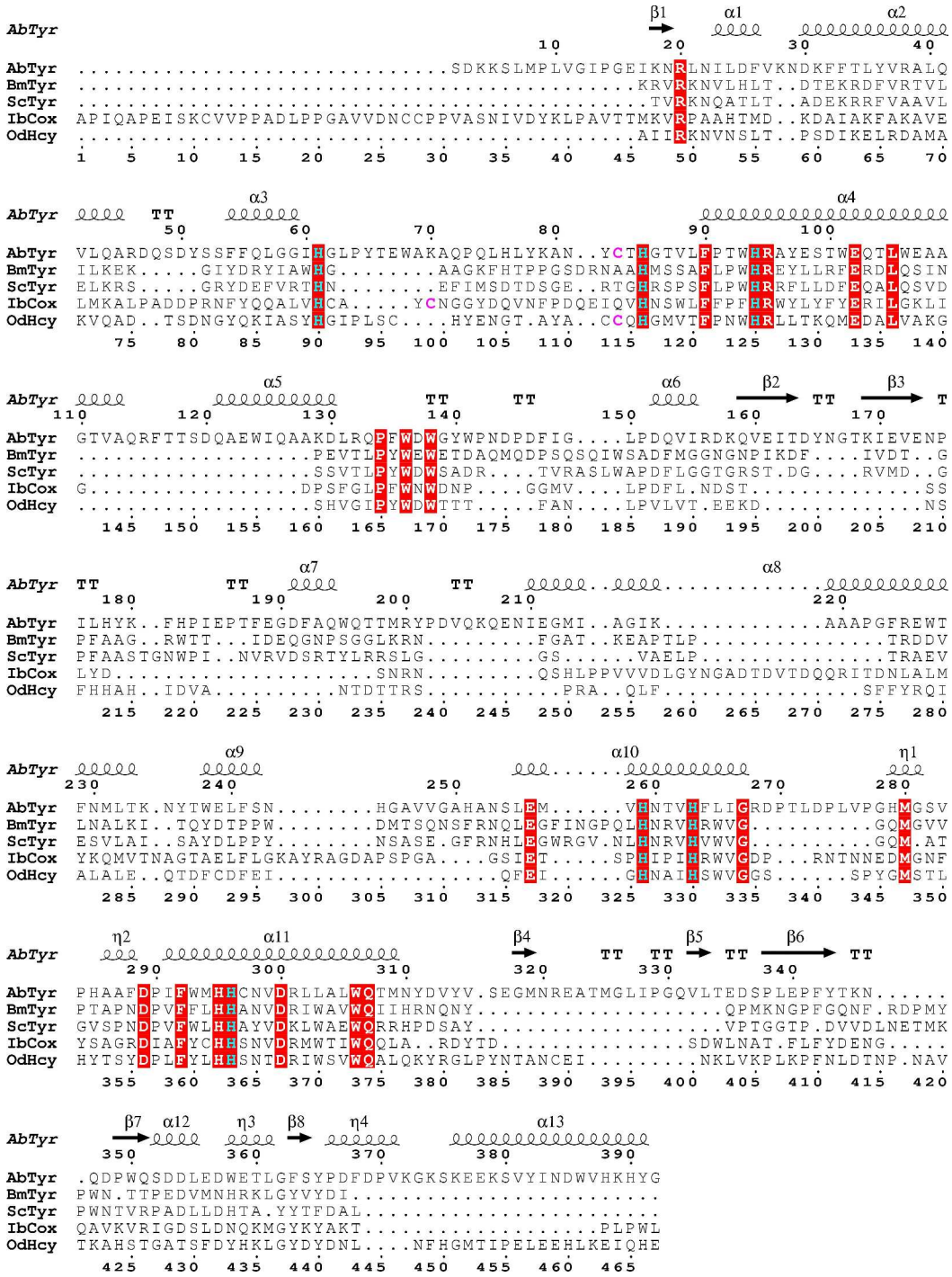


Fig. S3. Stereo figure of a superposition of *A. bisporus* tyrosinase (green; PDB 2y9w (this article)), *B. megaterium* tyrosinase (orange; PDB 3nq1 (*1*)), *S. castaneoglobisporus* tyrosinase (magenta; PDB 1wx2 (*2*)). Copper ions are in light brown and the inhibitor kojic acid from the *B. megaterium* tyrosinase structure is shown in orange.

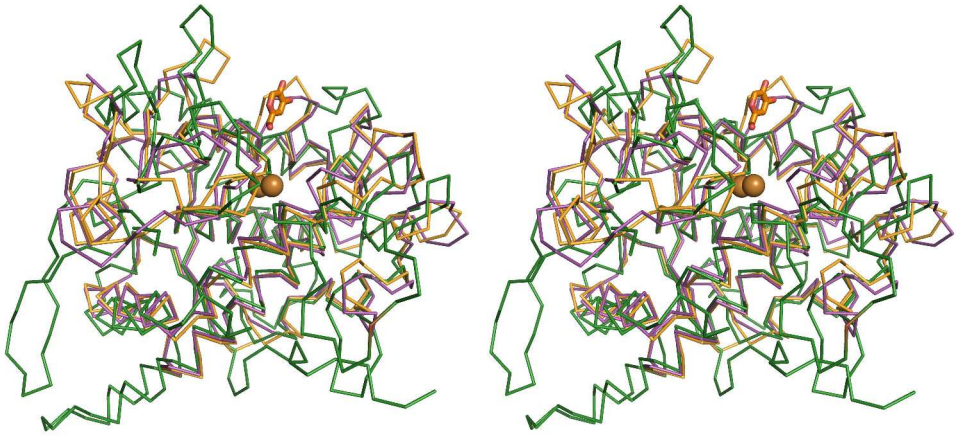
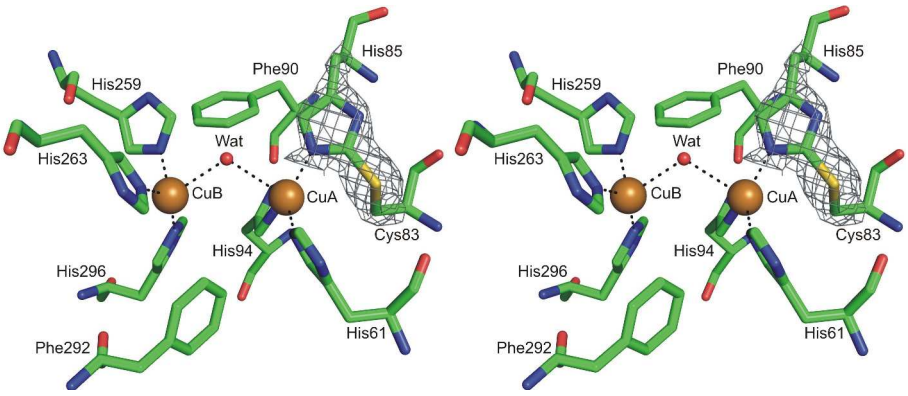


Fig. S4. Stereo figure of the geometry of the binuclear copper-binding site in space group $P2_12_12$. The ligands are colored according to atom type (green, blue, red, yellow for carbon, nitrogen, oxygen, sulfur, respectively). The brown spheres are the Cu-A and Cu-B copper ions, and the red sphere is a bound water molecule/hydroxyl ion. The electron density for the covalent thioether bond between the Cys83 Sγ and His85 Cε1 atoms is contoured at 2.0 σ and is shown in black mesh.



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